SPECIFICATION

Nucleic Acid Fragments, Recombinant Vectors Containing the Same and Method for Promoting Expression of Structural Genes Using the Same

Technical Field

Ins. A1

The present invention relates to a nucleic acid fragment having an activity to promote expression of a structural gene located at a downstream site thereof, a recombinant vector containing the same, and to a method for expressing the structural gene using the same, as well as to a plant in which expression of a desired structural gene is promoted by the method.

Background Art

Promotion of foreign gene expression is the most required technique in applying the genetic engineering technique to plants. One of the techniques is the utilization of DNA fragments. Known DNA fragments which promote expression of foreign genes include some introns (Simpson and Filipowicz 1996. Plant Mol.Biol. 32: 1-41) including an intron of maize alcohol dehydrogenase (Callis et al. Gene & Development 1, 1183-1200 (1987)), as well as the first intron of rice phospholipase D (WO96/30510). Influences of deletion of a part of inner regions of DNA fragments derived from introns, and of insertion of the same intron into the intron, on the promotion of expression have been reported (Mascarenhas et al. Plant Mol. Biol. 15, 913-920 (1990), Clancy et al. Plant Sci.98, 151-161 (1994)).

However, so far, types of available DNA fragments are limited. Further, actions of the DNA fragments vary depending on the type of the plant, and vary depending on the organs or tissues even in the same plant (Simpson and Filipowicz 1996. Plant Mol.Biol. 32: 1-41). Therefore, existence of DNA fragments exhibiting various types of expression-promotion actions is desired.

Disclosure of the Invention

Accordingly, an object of the present invention is to provide a novel nucleic

acid fragment having an activity to promote expression of a structural gene located downstream thereof, and to provide a method for promoting expression of the structural gene downstream thereof.

The present inventors intensively studied to discover that the second intron of rice PLD gene has a high activity to promote gene expression, thereby completing the present invention.

That is, the present invention provides a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, or having the same nucleotide sequence as shown in SEQ ID NO:1 except that one or more nucleotides are substituted or deleted, or one or more nucleotides are inserted therein or added thereto, which has an activity to promote expression of a structural gene located downstream thereof. The present invention also provides a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, or a nucleic acid fragment which hybridizes with the nucleic acid fragment under stringent conditions, which has an activity to promote expression of a structural gene located downstream thereof. The present invention further provides a recombinant vector which contains the above-described nucleic acid fragment according to the present invention and a structural gene located downstream of the nucleic acid fragment, by which expression of the structural gene is promoted by the nucleic acid fragment. The present invention further provides a method for promoting expression of a structural gene comprising inserting the nucleic acid fragment according to the present invention into a site upstream of the structural gene. The present invention further provides a plant in which expression of a desired structural gene is promoted, and progenies thereof retaining the character.

By the present invention, a novel nucleic acid fragment having a high activity to promote expression of a structural gene was provided. As is apparent from the Example below, the activity of the nucleic acid fragment according to the present

invention to promote expression of the structural gene downstream thereof is much larger than that of the known first intron of rice PLD gene, which has the similar function. Therefore, by inserting the nucleic acid fragment of the present invention into a site upstream of the structural gene, expression of the structural gene is much more promoted. Thus, by the present invention, for example, expression of a foreign gene using a recombinant vector may be much more promoted, so that the present invention will make a large contribution in the field of genetic engineering.

Best Mode for Carrying out the Invention

As mentioned above, the nucleic acid fragment according to the present invention is a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, or having the same sequence as shown in SEQ ID NO:1 except that one or more nucleotides are substituted or deleted, or one or more nucleotides are inserted therein or added thereto, which has an activity to promote expression of a structural gene located downstream thereof.

As mentioned above, the nucleic acid fragments (hereinafter also referred to as "modified nucleic acid fragment" for convenience) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which have activities to promote expression of a structural gene located downstream of the nucleic acid fragments are also within the scope of the present invention. In this case, the region in the modified nucleic acid fragment, which corresponds to a region in the sequence shown in SEQ ID NO:1 preferably has a homology of not less than 70%, more preferably not less than 85%, more preferably not less than 95% with the sequence shown in SEQ ID NO:1. The homology of the nucleotide sequence may easily be calculated by using a well-known software such as FASTA. Further, these modified nucleic acid fragments preferably hybridize with the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 1 under

stringent conditions (i.e., hybridization is carried out in an ordinary hybridization solution such as 5 x Denhardt's reagent, 6 x SSC, 0.5% SDS or 0.1% SDS, at 50 to 65°C, preferably in two steps at 50°C and at 60°C, or in four steps at 50°C, 55°C, 60°C and 65°C).

The nucleic acid fragments each of which is a part of the nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1, which have activities to promote expression of a structural gene located downstream of the nucleic acid fragments are also within the scope of the present invention. Further, nucleic acid fragments obtained by ligating a plurality of the nucleic acid fragments according to the present invention are also within the scope of the present invention. In this case, the nucleic acid fragments according to the present invention may be directly ligated or an intervening sequence may exist therebetween.

The nucleic acid according to the present invention may be either DNA or RNA. However, DNA is preferred in view of stability.

Since the nucleotide sequence of the nucleic acid fragment according to the present invention has been determined by the present invention and since the nucleic acid fragment is originated from the genome of rice, the nucleic acid fragment may easily be prepared by a nucleic acid-amplification method such as PCR using the genomic DNA of rice as the template. PCR is well-known in the art and a kit and apparatus therefor are commercially available, so that it can be easily carried out. Further, the above-mentioned modified nucleic acid fragments may be obtained by subjecting the thus obtained nucleic acid fragment to the well-known site-specific mutagenesis.

In cases where a plurality of nucleic acid fragments according to the present invention are ligated, a plurality of nucleic acid fragments according to the present invention may be preliminarily ligated, or a nucleic acid fragment according to the present invention may be inserted into a region containing the nucleic acid fragment

according to the present invention.

By inserting the above-described nucleic acid fragment according to the present invention to a site upstream of a structural gene, the expression of the structural gene may be promoted. Structural genes are controlled by a promoter located upstream thereof. The nucleic acid fragment according to the present invention may be inserted either between the promoter and the structural gene or at a site upstream of the promoter, and the former is preferred. In this case, the distance between the nucleic acid fragment according to the present invention and the structural gene may preferably be 0 bp to 1000 bp, and the distance between the promoter and the nucleic acid fragment according to the present invention may also preferably be 0 bp to 1000 bp.

The present invention also provides recombinant vectors obtained by applying the above-described method of the present invention to an expression vector. The recombinant vector according to the present invention may easily be prepared by inserting the nucleic acid fragment according to the present invention and a structural gene of which expression is to be promoted into a cloning site of a commercially available expression vector. Such an expression vector may preferably be one for plants. Various expression vectors for plants are well-known in the art and commercially available. These expression vectors include a replication origin for replication in host cells, a promoter, cloning sites giving restriction sites for inserting foreign genes, and a selection marker such as a drug resistant gene, and usually contain a terminator which stably terminates transcription. In the method of the present invention, any of these known expression vectors may be employed.

Example

The present invention will now be described more concretely by way of examples thereof. It should be noted that the present invention is not restricted to the Example.

A DNA fragment having the second intron of rice PLD gene and 37mer exon regions at both ends of the second intron (the nucleotide of this DNA fragment is shown in SEQ ID NO:2 in Sequence Listing) was amplified by PCR using the following primers and a known rice genomic clone (SEQ ID NO:5 of WO95/0934) as the template.

5'-aagtccccgggccgcgccagcggaag-3'

3'-gacaccgacagccgtctatagttcgta-5'

The obtained fragments amplified by PCR were digested with Sma I and Eco RV and inserted into the Sma I site of a vector pBI221 commercially available from CLONTECH, which contains a β-glucuronidase (GUS) gene at a downstream site of 35S promoter. Transient expression of the gene was examined by the method of Sheen (Sheen 1991, Plant Cell 3:225-245). That is, the constructed plasmids were introduced into protoplasts isolated from etiolated maize leaves by electroporation, and transient expression of GUS was measured by the above-mentioned method.

For comparison, the first intron (SEQ ID NO:3 in Sequence Listing) of rice PLD gene was amplified by PCR by the method described in WO96/30510 and inserted into the Sma I site of pBI221, followed by introduction of the obtained vector into maize in the similar manner as described above. The expression of GUS was determined. The results are shown in Table 1 below.

Table 1

Plasmid	GUS Activity (4-MU pmol/10^7 cells/min)
pBI221 (35S promoter, GUS)(Comparative Example)	140
pBI221 + PLD first intron (Comparative Example)	630
pBI221 + PLD second intron (Example)	11,000

As is apparent from these results, the activity of the nucleic acid fragment according to the present invention to promote expression of the structural gene located downstream thereof is much higher than that of the first intron of rice PLD, which is known to have the similar function.